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Bioavailability of phosphorus in granulated and pyrolyzed broiler manure

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ABSTRACT

Production of organic fertilizers from poultry manure may compromise availability of phosphorus (P) to plants. This study examined the effects of granulation, feather meal addition, and pyrolysis on bioavailability of P in broiler manure in a pot experiment with ryegrass and assessed whether P availability is enhanced by inoculating arbuscular mycorrhizal fungi (AMF) into soil. Granulated broiler manure gave similar plant yield and P uptake to superphosphate. Feather meal addition had a minor negative effect on P availability, whereas pyrolysis lowered the fertilization effect of broiler manure. The yield-based mineral-P equivalences were 120%, 85% and 75% during the first harvest, and 100%, 75% and 45% during one growing season for granulated unamended, granulated amended with feather meal and pyrolyzed broiler manure. Soil inoculation with AMF did not enhance P availability. Granulated poultry manure is suitable as a P fertilizer for annual crops with comparable bioavailability to mineral fertilizer P, whereas pyrolyzed poultry manure is suitable as a slow-release or storage P fertilizer in slightly acidic soils. Knowledge on P bioavailability in organic fertilizers produced with different technologies can be used for optimizing fertilization, minimizing build-up of soil P and its adverse environmental effects.

1. Introduction

Livestock production has become more intensive and regionally concentrated in recent decades, creating areas with high manure phosphorus (P) surpluses compared with crop requirements (Svanbäck et al., 2019; Ylivainio et al., 2014). This increases the risk of P accumulation in the soil, and subsequent P runoff and leaching. One option to mitigate this risk is to transport manure to areas requiring P, to replace mineral fertilizer use (Svanbäck et al., 2019). Poultry manure surpluses are increasing at present, as the poultry sector is expanding to meet the increasing demand for broiler meat and egg products (FAO, 2020). Adequately managed poultry manure is a valuable fertilizer that provides several essential nutrients for crops (Bolan et al., 2010), as well as organic matter, and can thus alleviate the declining organic matter content in agricultural soils (Heikkinen et al., 2013). However, the nitrogen:phosphorus (N:P) ratio of poultry manure is not optimal for crop requirements, and application rates based on crop N demand provide excess of P (Bolan et al.,

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2010). Additionally, challenges in logistics add to the transport costs (Paudel et al., 2009). This creates a need for manure processing technologies to better meet crop, environmental, and economic requirements and achieve optimal use of manure-based organic fertilizers.

Different processing technologies can be employed to produce nutrient-rich organic fertilizers with relatively high dry matter content from poultry manure. Common technologies such as composting and anaerobic digestion are beneficial for solids stabilization and for odor and pathogen reduction (Luostarinen et al., 2020). In addition, nutrient content in poultry manure can be optimized with appropriate co-feedstocks (Vandecasteele et al., 2014), but the ability of these technologies to concentrate nutrients is limited (Luostarinen et al., 2020). Instead, technologies with higher energy requirements, such as thermal drying, pelleting, and granulation, hygienize manure and concentrate nutrients more effectively (Luostarinen et al., 2020). Addition of ingredients to poultry manure prior to granulation or pelleting can produce fertilizer products with the desired nutrient content (Mazeika et al., 2016) but may simultaneously affect P bioavailability.

In recent years, thermochemical conversion technologies, such as slow pyrolysis, have been developed for converting various types of biomass to energy and carbonaceous end-products, while preserving and concentrating non-volatile nutrient elements (Mehta et al., 2015). Pyrolysis of manure produces alkaline hygienized char with a high ash content (Cantrell et al., 2012). It is known to transform P in manures to less soluble and bioavailable form (Huang et al., 2018), and thus pyrolyzed manures may be most suitable as a slow-release fertilizer (Wang et al., 2014). However, some studies on pyrolyzed poultry manure have reported increased or equal crop yield and P uptake compared with the unfertilized control (Subedi et al., 2016), while values comparable to inorganic P sources, such as water-soluble KH_2PO_4 (Rose et al., 2019) and single superphosphate (Duboc et al., 2017), have also been reported. When applied in soil, the solubility of P in recycled P fertilizers, especially those produced by thermochemical technologies, depends also on soil properties and particularly soil pH (Kratz et al., 2019). Therefore, further research is needed to clarify the P fertilizer value of pyrolyzed poultry manure.

One strategy to enhance P uptake from organic recycled fertilizers with limited P solubility could be to supply the soil with arbuscular mycorrhizal fungi (AMF), which form symbiotic relationships with crop roots (Smith and Read, 2008). The role of AMF symbiosis and its ability to enhance P acquisition by plants, especially on soils with low plant-available P concentration, is widely acknowledged (Johnson, 2010). In general, crops rely more on AMF for their nutrition in low-input agricultural systems (e.g., organic) than in conventional systems (Gosling et al., 2006; Kahiluoto et al., 2009). In previous studies, AMF have been found to enhance P uptake from organic fertilizers, such as bone meal (Kahiluoto and Vestberg, 1998) and compost (Cavagnaro, 2014), but little is known about their ability to acquire P from biochars. Although the majority of studies on AMF supplementation have focused on plant-derived biochars (Yang et al., 2020), studies by Hammer et al. (2014) and Solaiman et al. (2019) suggest that AMF-assisted P capture can enhance P availability from pyrolyzed manure. Therefore, AMF inoculation might be a promising way to increase P bioavailability from pyrolyzed poultry manure.

The aim of this study was to examine P bioavailability in a growth experiment of broiler manure-based organic fertilizers produced by two different technologies, granulation and pyrolysis, in comparison with superphosphate (SP). The effects on P bioavailability of N:P ratio adjustment with feather meal rich in plant-available N (Keskinen et al., 2020) and of AMF inoculation were also studied. The hypotheses tested were that: (1) bioavailability of P in granulated broiler manure is equal to that of P in mineral P fertilizer; (2) pyrolysis of broiler manure reduces readily bioavailable P content, but with time P is slowly released, maintaining constant P supply; and (3) soil inoculation with AMF enhances yield and P uptake by ryegrass from sparsely soluble P sources.

2. Materials and methods

2.1. Materials

Peat-bedded broiler manure (BM), granulated broiler manure (gBM, 3–6 mm), and granulated broiler manure spiked with feather meal (gBM-FM, 3–6 mm) were obtained from Biolan Ltd (Eura, Finland), a company producing organic soil amendments for horticultural purposes. The same batch of BM used for creating the granulated products was pyrolyzed at 460 °C (90 min), using a bench-scale pyrolysis device, in the laboratory at Luke Jokioinen. Four runs were required to obtain a sufficient amount of pyrolyzed manure (pyrBM, < 5 mm) for the pot experiment, for which batches were mixed on an equal mass basis. Mean yield (±SD) of the char, liquid, and gas fractions in pyrolysis was 37 (±0.0), 42 (±0.3), and 22 (±0.3) %, respectively, calculated from mass of air-dried and homogenized feedstock. Gas yield was calculated as the difference between feedstock mass and combined mass of the char and liquid fractions. The production of the BM products is described in more detail in Keskinen et al. (2020).

Commercial AMF inoculum (SR1 for field vegetables and pulses) was acquired from Plantworks Ltd (UK). It contained spores, mycelium, and dried root fragments colonized by mycorrhizal fungi from the genera *Funneliformis*, *Claroideoglomus*, and *Rhizophagus*, together with a biostimulant containing molasses and plant-derived amino acids.

2.2. Pot experiment

A pot experiment with Italian ryegrass (*Lolium multiflorum*, var. Barmultra II) was established outdoors under a glass roof at ambient air temperature in Jokioinen, Finland (60°48′42″N, 23°28′48″E) in summer 2018, and was run for four months. The experimental period was unusually warm, with local heat sum as growing degree days (5 °C threshold, 14 April–22 Oct. 2018) of 1730, compared with a long-term (1981–2010) average of 1320. The experiment comprised the following treatments: (1) gBM, (2) gBM-FM, (3) gBM-FM+AMF, (4) pyrBM, (5) pyrBM+AMF, and five levels of SP for obtaining yield response curves.

The experimental soil was a sandy soil from Mikkeli, eastern Finland (Keskinen et al., 2020). The soil had low soil test P concentrations of 2.0 mg L^{-1} for acidic (pH 4.65) ammonium acetate extractable P (Vuorinen and Mäkitie, 1955), 17 mg kg^{-1} in dry matter (DM) for Olsen P (Olsen and Sommers, 1982) and 16 mg L^{-1} for Mehlich-3 P (Mehlich, 1984). The soil was air-dried and sieved (14 mm sieve) to remove coarse fragments and root debris, after which 6.0 kg soil were weighed into each pot (diameter 22 cm, volume 6.5 L) and limed with $Ca(OH)_2$ (9.1 g pot^{-1}) to achieve pH 6.5 (original pH was 6.1; water, 1:2.5 v/v). All pots received the following amounts of nutrients (mg pot^{-1}), to ensure that P would be the only limiting nutrient: 1500 N (NH_4NO_3, $Ca(NO_3)_2$), 2000 K (KCl), 300 Mg ($MgSO_4$), 450 S (sulfate salts and H_2SO_4), 6000 Ca (lime, $Ca(NO_3)_2$), 20 Fe ($FeSO_4$), 20 Zn ($ZnSO_4$), 20 Mn ($MnSO_4$), 10 Cu ($CuSO_4$), 2 B (H_3BO_3), and 2 Mo (Na_2MoO_4).

Various P sources per pot were applied at rates based on their total P concentration (Table 1), to achieve 100 mg P kg^{-1} soil (600 mg P pot^{-1}) at the higher P level treatments (gBM, gBM-FM, gBM-FM+AMF, pyrBM, pyrBM+AMF) and 30 mg P kg^{-1} soil (180 mg pot^{-1}) at the lower P level treatments (gBM-FM and gBM-FM+AMF). Application rates for the SP treatments were 0, 25, 50, 150, and 300 mg P kg^{-1} soil (treatments SP0, SP25, SP50, SP150, SP300). Lime was mixed into the whole soil volume, whereas nutrients and P sources were mixed after separating approximately 0.3 L sieved (4 mm) soil needed for covering the seeds.

The AMF-treated pots were established similarly to the untreated pots, except that 10 mL granular (1–3 mm) SR1-product (over 10-fold the recommended field dose) were scattered evenly over the soil surface and covered with a 2-cm layer of the fertilized and limed soil.

On the following day (30 May), each pot was sown with 0.3 g seeds (∼1 germinating seed per 4 cm^2) and covered with unfertilized limed soil. Before sowing, the pots were watered with 0.5 L deionized water. During the growing period, the pots were drip-irrigated with deionized water according to evapotranspiration. Ryegrass was harvested four times by cutting at 2 cm above the soil surface. The first harvest was carried out 36 days after the sowing and the subsequent harvests 20, 28, and 33 days after the previous harvest. Following the first and second harvests, 1500 mg of N and K were applied to all pots, while after the third harvest 1000 mg of N and K were added per pot to ensure sufficiency for the following yield.

2.3. Laboratory analyses

2.3.1. Products

Bulk density of the products was determined (SFS-EN 13040) prior to milling. Then gBM, FM, and gBM-FM were milled to pass through a 2 mm sieve, and pyrBM to pass through a 1 mm sieve, before laboratory analyses. Dry matter content at 103 °C and ash content as loss on ignition at 550 °C were determined using a LECO TGA-701 analyzer. Sample pH was determined on 1:5 (v/v) solid-to-water suspensions (SFS-EN 13037) and, after filtering through Whatman 40 paper, electrical conductivity (EC) was determined (SFS-EN 13038). After *aqua regia* digestion (SFS-EN 13650), total concentrations of Al, Fe, P, K, Ca, Mg, Mn, S, Cu, Ni, and Zn were analyzed by ICP-OES (Perkin Elmer Optima 8300), and those of As, Cd, and Pb by graphite furnace AAS (Varian AA280Z). Total N and C concentrations were determined by the Dumas method (LECO TruMac CN -analyzer; LECO Corporation, St. Joseph, MI, USA). The commercial SP fertilizer used as reference was also included in the total element analyses, after milling to pass through a 2 mm sieve.

Solubility of P in the BM products (all milled to pass through a 1 mm sieve) was assessed according to a modified Hedley fractionation scheme containing the following sequential extractions (1:60, w/v): two consecutive water extractions, followed by extractions with 0.5 *M* $NaHCO_3$, 0.1 *M* NaOH, and 1 *M* HCl (Sharpley and Moyer, 2000). Duration was 4 h for the first water extraction, or otherwise 16 h. All products (1 g DM) were moistened with deionized water (5 mL; volume subtracted from the first water extraction) one week before fractionation, to reduce hydrophobicity. After each extraction step, the samples were centrifuged (3000×*g*, 15 min) and, for determination of inorganic P, filtered through a 0.2 μm Nuclepore membrane (Whatman, Maidstone, UK). For total P determination, unfiltered supernatants (except for the HCl fraction) were digested at 120 °C with sulfuric acid and peroxodisulfate. Concentrations in the supernatants were analyzed spectrophotometrically (Shimadzu UV-160 A) according to Murphy and Riley (1962). Organic P (Po) was calculated as the difference between total P and inorganic P (Pi). The fraction of labile P was calculated as the sum of water- and $NaHCO_3$-soluble P, containing both organic and inorganic P fractions. Residual P was calculated by subtracting the sum of the fractions from *aqua regia*-soluble P.

Laboratory analyses for gBM, FM, gBM-FM, and SP were performed on triplicate samples, while for pyrBM each of the four pyrolysis batches was analyzed separately.

Table 1
Total concentrations (mean ± SD) of nutrients, iron, aluminum, carbon, ash, and dry matter (DM), and values of pH, electric conductivity (EC), bulk density, N:P ratio, and molar ratios of Ca:P and (Fe + Al):P, in granulated broiler manure (gBM), gBM spiked with feather meal (gBM-FM), pyrolyzed broiler manure (pyrBM), feather meal (FM), and superphosphate (SP) (n = 3, except for pyrBM n = 4).

	Material				
	gBM	gBM-FM	pyrBM	FM	SP
DM (%)	89 ± 0.01	89 ± 0.05	99 ± 0.02	94 ± 0.02	96 ± 0.05
Ash (%)	14 ± 0.09	12 ± 0.03	35 ± 0.64	3.5 ± 0.21	na[a]
Bulk density (kg m^{-3})	600 ± 1.8	570 ± 4.1	370 ± 8.3	570 ± 2.3	na[a]
pH	6.2 ± 0.01	5.9 ± 0.01	11 ± 0.07	5.9 ± 0.02	na[a]
EC (mS cm^{-1})	12 ± 0.12	9.6 ± 0.07	7.5 ± 0.21	2.1 ± 0.01	na[a]
P (g kg^{-1} DM)	11 ± 0.00	8.8 ± 0.17	25 ± 0.26	2.8 ± 0.08	97 ± 2.0
K (g kg^{-1} DM)	28 ± 0.26	20 ± 0.26	66 ± 1.0	1.4 ± 0.04	2.6 ± 0.62
Ca (g kg^{-1} DM)	17 ± 0.26	14 ± 0.31	44 ± 1.6	4.4 ± 0.21	210 ± 4.0
Mg (g kg^{-1} DM)	7.6 ± 0.06	5.6 ± 0.10	20 ± 0.28	0.31 ± 0.00	4.8 ± 0.12
Mn (g kg^{-1} DM)	0.57 ± 0.01	0.41 ± 0.01	1.5 ± 0.05	0.02 ± 0.00	0.16 ± 0.01
S (g kg^{-1} DM)	7.1 ± 0.07	9.3 ± 0.19	10 ± 0.23	17 ± 0.10	140 ± 2.0
Fe (g kg^{-1} DM)	1.4 ± 0.02	1.1 ± 0.01	3.2 ± 0.08	0.08 ± 0.00	1.6 ± 0.16
Al (g kg^{-1} DM)	0.44 ± 0.02	0.32 ± 0.00	1.2 ± 0.02	0.01 ± 0.00	1.5 ± 0.05
N (% DM)	4.3 ± 0.02	7.0 ± 0.10	4.5 ± 0.03	15 ± 0.03	0.23 ± 0.03
C (% DM)	43 ± 0.11	46 ± 0.05	54 ± 0.26	53 ± 0.09	0.11 ± 0.00
N:P	4.0 ± 0.02	8.0 ± 0.14	1.8 ± 0.02	53 ± 1.5	0.02 ± 0.00
Ca:P, molar ratio	1.2 ± 0.02	1.2 ± 0.00	1.3 ± 0.05	1.2 ± 0.02	1.7 ± 0.03
(Fe+Al):P, molar ratio	0.12 ± 0.00	0.11 ± 0.00	0.12 ± 0.00	0.02 ± 0.00	0.03 ± 0.00

[a]Not analyzed.

2.3.2. Plant analyses

The harvested ryegrass was dried at 60 °C for DM yield determination and thereafter milled in a hammer mill (< 1 mm) for element analyses. Concentrations of P, Ca, Cu, Fe, K, Mg, Mn, S, and Zn were analyzed after HNO_3 digestion (0.5 g plant material with 10 mL 70% HNO_3) with ICP-OES (Perkin Elmer Optima 8300). Concentration of N in plant material was determined by near infrared spectroscopy (BÜCHI NIRFlex N500, Switzerland).

2.3.3. AMF

After the last harvest, roots of one plant from each pot were gently separated from the soil for assessment of AMF colonization. The roots with the soil attached were stored in plastic bags overnight at 4 °C and then rinsed with tapwater and stored in 50 mL Falcon tubes in 60% ethanol for 1–2 weeks at 4 °C prior to staining.

To assess AMF colonization, the ryegrass roots were stained with the ink and vinegar method (Vierheilig et al., 1998). In brief, the roots were incubated in 10% KOH for 72 h at room temperature, kept for 10 min at 60 °C in 5% Sheaffer Skrip Black ink, and rinsed with 5% vinegar. Stained roots were stored at 4 °C in lactic acid, glycerol, and water (1:1:1). Thereafter, 15 pieces of 10-mm root sections were mounted on glass slides. For each sample two slides were made, one of thicker roots (main root and laterals) and one of thin outermost root branches and root tips. Mycorrhizal colonization (hypha, arbuscules, and vesicles) was assessed by the gridline-intersection method (McGonigle et al., 1990) from a total of 2×105 field views per sample, under compound microscope (10× magnification). If hypha was present alone or attached to arbuscules or vesicles, the field view was counted as mycorrhizal. The number of mycorrhizal views was divided by the total number of field views to determine the colonization percentage. The percentages for arbuscules and vesicles were determined in a similar manner.

2.3.4. Soil

After AMF sampling, the remaining roots and plant materials were removed by hand and the soil was thoroughly mixed and sampled (300 mL). The soil samples were dried at 37 °C in a ventilated oven and milled (< 2 mm) for analysis of acidic ammonium acetate-extractable P (STP) and pH (water, 1:2.5 v/v) according to Vuorinen and Mäkitie (1955), which was performed at Eurofins Agro Finland.

2.4. Mineral-P equivalence

To estimate bioavailability of P, DM yield and P uptake of ryegrass in the BM treatments were compared against yield and P uptake response curves produced by SP. For the first and total yield (sum of four yields), yield mass response to increasing SP additions was fitted to the equation: $Y = A + B\times(1 - e^{-Cx})$ to solve parameter values for A, B and C. Parameter A is the yield obtained without P addition (y-axis intercept), B is the maximum yield increase obtained with P, e is Euler's number, C is a soil specific fitting parameter, and x is the amount of added P (mg kg^{-1} soil). The same equation was then used to calculate P uptake (yield mass × P concentration) as response variable. Mineral-P equivalence of the BM products was deduced by comparing the amount of SP phosphorus (SP-P) required to obtain similar yield or P uptake as recorded for the BM products. Equivalence was expressed as percentage of total P added in the BM product. Short-term

P bioavailability was determined from the first harvest. Apparent P recovery (%) was calculated by subtracting P uptake in the SP0 treatment from P uptake in the BM treatment and relating it to total amount of P added.

2.5. Statistical analysis

The pot experiment was carried out according to a completely randomized block design, with four harvests and blocks. To test the effects of the BM products on yield and P uptake in each harvest, a linear mixed model (LMM) (restricted maximum likelihood estimation method; REML) for yield and generalized linear mixed model (GLMM) with Gamma distribution (residual pseudo-likelihood estimation method; REPL) for P uptake were used. BM product, harvest, and their interaction were treated as fixed effects, and block and its interaction with harvest as random effects. Correlation between harvests was taken into account using either heterogeneous first-order autoregressive or heterogeneous compound symmetry covariance structure.

Differences in total yield and P uptake, STP, and pH were analyzed with LMM (REML method), with BM product as fixed effect and block as random effect. Differences in total yield and P uptake between the BM products were analyzed, whereas SP300 was excluded from the STP analyses, and SP25, SP50, SP150, and SP300 were excluded from the soil pH analyses, to minimize pairwise comparisons. For total yield, variance differed between the BM products.

Mycorrhizal colonization and abundances of arbuscules and vesicles in thin and thick roots were analyzed with GLMM. The assumption of binomial distribution (REPL) was used, with BM product, root class (thin or thick roots), and their interaction as fixed effects and block as random effect. Correlation between different root classes was taken into account using compound symmetry covariance structure.

The Tukey method (Westfall et al., 2011) was used for pairwise comparisons of means (significance level α =0.05) and the Kenward–Roger method (Kenward and Roger, 2009) for calculating degrees of freedom. Suitability of all models was studied based on normality of residuals, confidence limits, and comparing the estimates to mean data values. The analyses on total yield, P uptake, STP values, and soil pH were performed using the MIXED procedure, and those on individual harvests, mycorrhizal colonization, and abundances of arbuscules and vesicles using the GLIMMIX procedure in the SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Characteristics of the P sources

The characteristics of the BM products studied varied widely (Table 1). As non-volatile elements are concentrated to the char fraction in pyrolysis, total P, K, Ca, Mg, and Mn concentrations were highest in pyrBM, two- to three-fold higher than in gBM and gBM-FM. Pyrolyzed BM had also the highest pH, while total C concentration was similar to that in FM and total N concentration was similar to that in gBM. Trace element concentrations present are reported by Keskinen et al. (2020), who studied plant availability of N in the same products. Trace element concentrations in all BM products were below the EU statutory thresholds for organic fertilizer products (2019/1009), with the exception of Zn in pyrBM (1100 vs. 800 mg kg^{-1} DM).

Based on the Hedley fractionation results, most of the P in all BM products was inorganic (Table 2). Phosphorus in gBM and gBM-FM was mainly labile (60%), and primarily water-soluble. The proportions of acid-soluble and residual P were low. In contrast, P in pyrBM was mainly acid-soluble and residual, whereas the content of labile P was moderate (26%) and mainly $NaHCO_3$-soluble. However, on an equal mass basis, the amount of labile P in pyrBM (6.6 g kg^{-1} DM) was comparable to that in gBM (6.4 g kg^{-1} DM) and higher than in gBM-FM (5.5 g kg^{-1} DM). Phosphorus content in FM used in production of gBM-FM was moderate (Table 1), with nearly equal proportions of water-soluble, and residual and acid-soluble P (Table 2).

3.2. Colonization of ryegrass roots by AMF by the end of the experiment

Mycorrhizal colonization (42%–83%) and abundances of arbuscules (9.9%–74%) and vesicles (15%–38%) in ryegrass roots after the fourth harvest were found to be independent of AMF inoculation, soil P level, and BM product (Supplementary material, Table S1). Degree of colonization and abundances of arbuscules and vesicles were higher (colonization: 21%-unit, $p < 0.001$, arbuscules: 35%-unit, $p < 0.001$, vesicles: 9%-unit, $p = 0.010$) for thin roots compared with thick roots. The highest degree of colonization (83%) was observed for the thin outermost lateral roots/root tips of ryegrass in the gBM-FM treatment at the higher P level.

3.3. Ryegrass yield

At the first harvest, ryegrass yield was low and varied considerably between replicates, due to slow onset of growth. Throughout the experiment, the highest yield among the BM products was in the gBM treatment (Fig. 1). At the first harvest, yield in gBM was 52% higher ($p = 0.002$) than in the pyrBM treatment. At the second and third harvests, gBM produced 35% ($p < 0.001$) and 26% ($p = 0.002$) higher yields than gBM-FM and 66% ($p < 0.001$) and 25% ($p = 0.003$)

Table 2
The shares of inorganic (Pi), organic (Po), and residual phosphorus (% in dry matter; mean± SD) in granulated broiler manure (gBM), gBM spiked with feather meal (gBM-FM), pyrolyzed broiler manure (pyrBM), and feather meal (FM), based on the Hedley fractionation scheme (n = 3, except for pyrBM n = 4).

Extractant		%			
		gBM	gBM-FM	pyrBM	FM
Water	Pi	49 ± 0.36	51 ± 1.1	5.8 ± 0.30	32 ± 1.8
	Po	8.1 ± 0.26	9.7 ± 0.79	0.77 ± 0.20	15 ± 0.74
$NaHCO_3$	Pi	1.9 ± 0.11	1.9 ± 0.02	18 ± 0.34	2.2 ± 0.28
	Po	0.95 ± 0.21	1.0 ± 0.31	1.1 ± 0.25	3.0 ± 0.49
NaOH	Pi	3.5 ± 0.06	2.8 ± 0.01	4.8 ± 0.12	0.39 ± 0.06
	Po	7.8 ± 1.9	7.6 ± 0.34	0.52 ± 0.10	0.20 ± 0.12
HCl	Pi	13 ± 0.31	16 ± 0.39	48 ± 1.3	34 ± 2.9
$\sum$	Pi	68 ± 0.66	72 ± 1.5	77 ± 1.4	69 ± 4.5
	Po	17 ± 2.0	18 ± 1.0	2.4 ± 0.43	18 ± 1.1
Residual		15 ± 1.7	10 ± 2.2	21 ± 1.2	13 ± 5.0

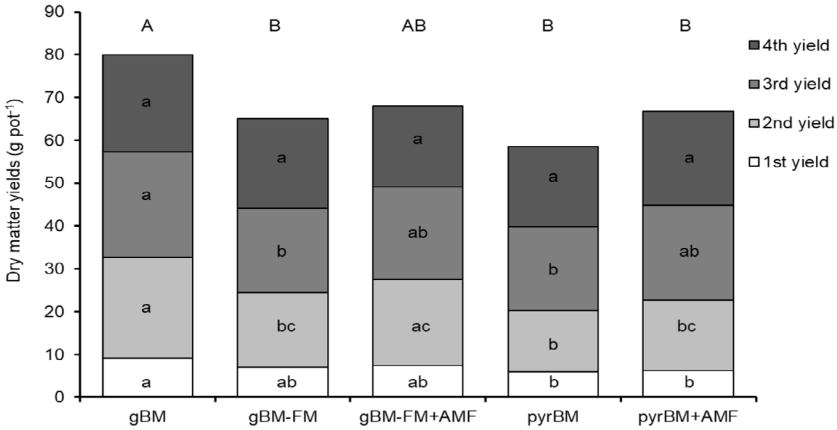

Fig. 1. Dry matter yield (g pot^{-1}) of ryegrass grown with the different broiler manure products (n = 4) at the higher P level (100 mg P kg^{-1} soil). The results are mean estimates. Statistically significant differences at chosen significance level ($p < 0.05$) in individual yield between the products are marked with different lower case letters, and differences in total yield with different capital letters. Abbreviations: gBM = granulated broiler manure; gBM-FM = gBM spiked with feather meal; pyrBM = pyrolyzed BM; AMF = arbuscular mycorrhizal fungi.

higher yields than pyrBM. At the fourth harvest, yield differences between the BM products leveled off. Yield in the pyrBM treatment increased from first to third harvest, whereas yield in the other BM treatments leveled off from the second harvest onwards. Total yield was 23% ($p = 0.050$) higher in gBM than in gBM-FM and 36% ($p = 0.019$) higher than in pyrBM treatment. No clear yield effect by AMF inoculation of soil was detected at either the higher (Fig. 1) or the lower P level. At the higher P level, total yields were in inoculated treatments 5% ($p = 0.957$) and 14% ($p = 0.263$) higher than in uninoculated gBM-FM and pyrBM treatments, respectively. At the lower P level, there was no difference (−1.2%, $p = 1.000$).

3.4. Phosphorus uptake

Phosphorus uptake between gBM and gBM-FM followed the similar pattern as in yields. Phosphorus uptake in the gBM treatment was 30% ($p = 0.378$), 13% ($p = 0.940$), 21% ($p = 0.191$) and 6% ($p = 0.884$) higher than in gBM-FM treatment in the consecutive harvests, and 15% ($p = 0.139$) higher in total (Fig. 2). However, total P uptake in gBM was 53% ($p < 0.001$), and that in gBM-FM 33% ($p = 0.005$) higher, than in the pyrBM treatment. At the two first harvests, P uptake in gBM (122% and 130%, $p < 0.001$) and gBM-FM (first harvest: 70%, $p = 0.003$; second harvest: 104%, $p < 0.001$) was higher than in the pyrBM treatment, after which the differences leveled off. A similar pattern was observed in shoot P concentrations (Supplementary material, Table S2). For all BM products except pyrBM, the highest P uptake occurred at the second harvest, while uptake in the pyrBM treatment peaked at the third harvest. No clear effect on P uptake by AMF inoculation was detected at either the higher (Fig. 2) or the lower P level. At the higher P level, total P uptake was in inoculated treatments 12% ($p = 0.364$) lower than in gBM-FM and 9% ($p = 0.865$) higher than in pyrBM. At the lower P level, there was no difference (2%, $p = 1.00$).

Mean apparent P recovery was highest in the gBM treatment (23 ± 1.2%) followed by gBM-FM (19 ± 1.9%) and pyrBM (13 ± 2.8%). Apparent P recovery was not affected by AMF inoculation at either the higher (gBM-FM+AMF: 16 ± 3.7%, pyrBM+AMF: 15 ± 1.7%) or lower P level (gBM-FM: 20 ± 3.0%, gBM-FM+AMF: 21 ± 1.9%). Recovery in the SP25, SP50,

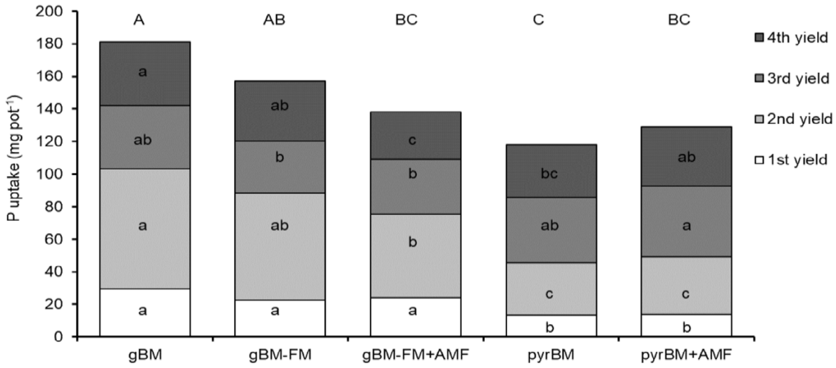

Fig. 2. Phosphorus uptake (mg pot^{-1}) by ryegrass grown with different broiler manure products (n = 4) at the higher P level (100 mg P kg^{-1} soil). Statistically significant differences at chosen significance level ($p < 0.05$) in individual yield between the products are marked with different lower case letters and differences in total yield with different capital letters. Abbreviations: gBM = granulated broiler manure; gBM-FM = gBM spiked with feather meal; pyrBM = pyrolyzed BM; AMF = arbuscular mycorrhizal fungi.

SP150, and SP300 treatments was 27 ± 6.3, 24 ± 4.6, 16 ± 0.6, and 11 ± 0.5%, respectively. During the whole study period, shoot N:P ratio in the BM treatments varied from 11 to 40 (Supplementary material, Table S2).

3.5. Mineral-P equivalence

Mineral-P equivalence based on P uptake (Supplementary material, Figs. S1 and S2) was roughly consistent with that based on yield (Figs. 3 and 4), although in the gBM and gBM-FM treatments the P uptake (total)-based equivalence was 35% and 80% higher, respectively, than the corresponding yield-based values. Only yield-based equivalence is reported here, due to the practical context in agriculture.

Yield-based short-term (first harvest) equivalence in the gBM and gBM-FM treatments varied widely at the higher P level, due to individual replicates with much higher yield than in other pots (Fig. 3). The short-term equivalence of gBM, gBM-FM, and pyrBM was 150 ± 60%, 96 ± 46%, and 74 ± 18%, respectively. Excluding the deviating replicates, the equivalence of gBM and gBM-FM was 120 ± 12% and 74 ± 16%, respectively. Based on total yield, the mineral-P equivalence of gBM (100 ± 7.7%) was equal to that of SP, while the mineral-P equivalence of gBM-FM was 58 ± 12% and that of pyrBM treatment 45 ± 11% (Fig. 4). However, at the lower P level, the short-term and total yield-based mineral-P equivalences for gBM-FM were 93 ± 16% and 88 ± 31%, respectively. No clear effect on the equivalence by AMF inoculation was detected at either the higher or lower P level. The short-term equivalence at the higher P-level was 100 ± 17% for gBM-FM+AMF and 79 ± 4.8% for pyrBM+AMF, and at the lower P level 91 ± 16% for gBM-FM+AMF. Based on total yield the equivalence at the higher P level was 65 ± 12% for gBM-FM+AMF and 61 ± 3.2% for pyrBM+AMF, and at the lower P level 84 ± 7.9% for gBM-FM+AMF.

3.6. Soil test P and pH at the end of the experiment

In all BM treatments at the higher P level, the STP values at the end of the experiment exceeded ($p < 0.001$) that in the unfertilized control (2.2 mg L^{-1}). The values in the gBM (3.4 mg L^{-1}), gBM-FM (3.6 mg L^{-1}), and pyrBM (3.3 mg L^{-1}) treatments were comparable to that in the SP150 treatment (3.6 mg L^{-1}). Soil pH at the end of the experiment was at the same level in the unfertilized control (5.6) and gBM-FM treatment (5.7), and somewhat higher ($p < 0.001$) in the gBM (6.0) and pyrBM (6.1) treatments. The STP and pH values were not affected by AMF inoculation at either the higher or the lower P level (data not shown).

4. Discussion

The P solubility values acquired by Hedley fractionation and the growth responses in the pot experiment both ranked the BM products tested in the same order regarding their P bioavailability. The P bioavailability of gBM was comparable to, or even slightly higher than, that of the SP treatment, including in the beginning of the growth period, supporting our first hypothesis. Similarly, a field study by Sneller and Laboski (2009) demonstrated that P in dehydrated and pelleted poultry manure from laying hens fed a phytase-amended diet was equally available to corn (*Zea mays* L.) as P in mineral P fertilizer. In contrast, Delin (2016) found that mineral-P equivalence of fresh non-granulated chicken manure in a 11-week pot experiment with two harvests of ryegrass was only 64%. The lower value compared with our study might be a consequence of fewer harvests and different timing of manure application. The apparent P recovery of 23% in our study was higher than reported for pelleted poultry manure for corn in field studies (7.5% during the first growing season) by

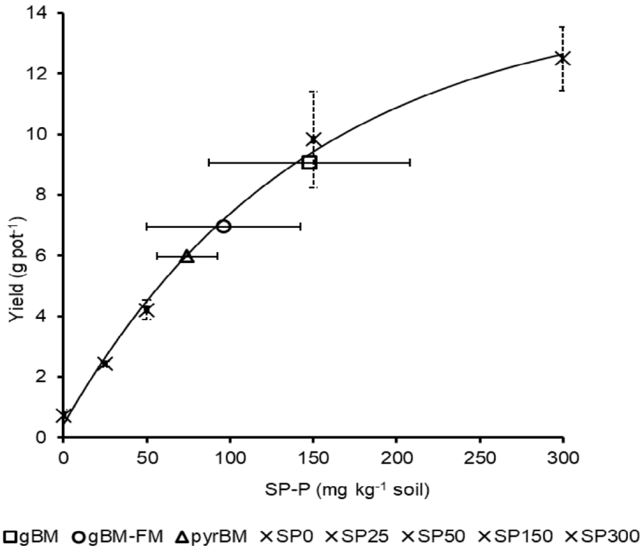

Fig. 3. Short-term (first harvest) growth response of ryegrass (mean with SD bars) to superphosphate phosphorus (SP-P) and amount of SP-P (mean with SD bars) required to obtain similar yield with granulated broiler manure (gBM), gBM spiked with feather meal (gBM-FM), and pyrolyzed broiler manure (pyrBM) at the higher P level of 100 mg kg^{-1} soil (n = 4). Mineral-P equivalences of the broiler manure products are derived from the SP-P values as percentage of total P added.

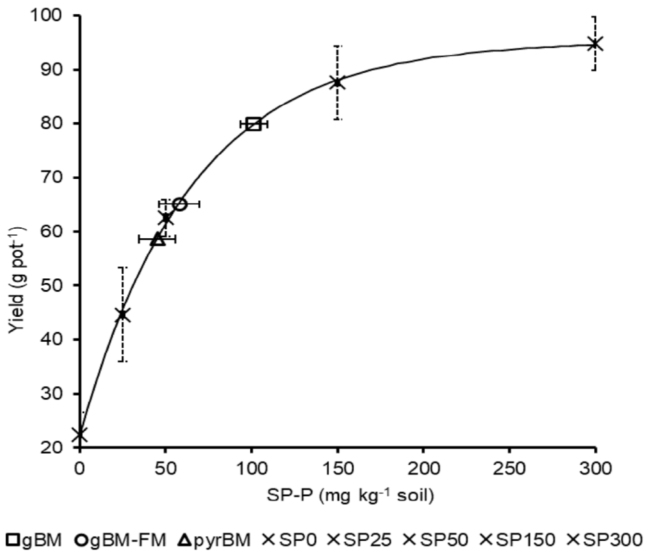

Fig. 4. Cumulative (sum of four harvests) growth response of ryegrass (mean with SD bars) to superphosphate phosphorus (SP-P) and amount of SP-P (mean with SD bars) required to obtain similar yield with granulated broiler manure (gBM), gBM spiked with feather meal (gBM-FM), and pyrolyzed broiler manure (pyrBM) at the higher P level of 100 mg kg^{-1} soil (n = 4). Mineral-P equivalences of the broiler manure products are derived from the SP-P values as percentage of total P added.

Sneller and Laboski (2009) and for millet (*Urochloa ramosa* (L.)) in mineral soils in pot experiments (9.6%–19%; calculated using data in (Montalvo Grijalva et al., 2010).

Higher P bioavailability in manures compared with mineral fertilizers has also been observed in previous studies (Ylivainio et al., 2021). High P bioavailability of gBM is probably associated with high solubility of Ca phosphates in gBM in slightly acidic soil as low molar ratio of (Fe+Al):P (Table 1) does not predict P bioavailability (Ylivainio et al., 2021). Overall, granulation of poultry manure yields fertilizer with good P bioavailability, providing P sufficiently also during the early growth stage.

While gBM can serve as a good P source, it provides an insufficient amount of N for crops. In this study, we tested a more balanced fertilizer produced by mixing FM with broiler manure as an additional N source (for N plant availability, see Keskinen et al., 2020). Although the results at the higher P level suggested a decrease in yield-based mineral-P equivalence for gBM-FM, the yield-based equivalences at the lower P level were close to that of SP. In addition, the P uptake-based equivalences at the higher P level were comparable to mineral fertilizer, being accordance with P solubility (Table 2) and indicating that the reduced yields were not due to low P availability. Ryegrass yield was not affected by deficiency of other nutrients, since the shoot concentrations of Ca, Cu, Fe, K, Mg, Mn, S and Zn were within the adequate range (Bergmann, 1992, Supplementary material, Table S2). To conclude, P availability of gBM-FM is likely comparable to that of mineral fertilizer.

Pyrolyzed broiler manure produced lower ryegrass yields than gBM, as expected from the lower P solubility in Hedley fractionation. Reduced P solubility in manure due to pyrolysis is a known effect, as the relative abundance of less soluble Ca phosphates increase as pyrolysis temperature rises (Huang et al., 2018). According to Vanden Nest et al. (2021) the P bioavailability of biochars produced at higher temperatures from plant biomass and pig manure decreases with increasing Ca:P molar ratio. In the present study, the decline in P solubility in pyrBM led to P deficiency (indicated by shoot N:P ratio >20; see Güsewell, 2004; Supplementary material Table S2) and poorer ryegrass growth than in gBM during the whole growing period. In comparison, gBM and gBM-FM provided sufficient P to ryegrass for much longer, as P deficiency was not observed until the third harvest. As the growing season progressed, pyrBM tended to increase yields relative to the other products, supporting our second hypothesis that slow release P from pyrBM enables constant P supply. However, P availability in manure biochars is largely controlled by soil pH, as Ca phosphates are more soluble in acidic conditions (Kratz et al., 2019). For example, in a pot experiment by Subedi et al. (2016) the apparent P recovery values for poultry litter biochars (400 °C) were 6.7% and 21% in moderately alkaline (pH 8.3) sandy and slightly acidic (pH 6.1) silt-loam soil, respectively. In the present study, the soil was limed to the target pH of 6.5 which, together with a possible neutralizing effect of the pyrBM, may have reduced P solubilization from pyrBM leading to moderate P availability. However, at the end of the experiment, soil pH in the pyrBM treatment was comparable to that in the gBM treatment. Despite our study only lasting one growing season, the results support the hypothesis that pyrolysis of poultry manure is suitable for making slow-release P fertilizer products for slightly acidic soils.

To enhance P availability of the pyrBM and gBM-FM, commercial AMF inoculum was added, and plant yield and P uptake were compared with those for soil without added AMF. It emerged that the soil used in the pot experiment contained indigenous AMF, which led to AMF colonization of ryegrass roots regardless of the inoculation. In fact, AMF inoculum is often outcompeted by native soil AMF that are more adapted to local soil conditions (Gosling et al., 2006). Inoculation did not improve ryegrass yield or P uptake compared with the non-inoculated treatments, contradicting our third hypothesis. This is consistent with findings by Kahiluoto and Vestberg (1998) that AMF inoculum did not increase yield or P uptake by leek (*Allium porrum* L.) from bone meal compared with native AMF, and even slightly decreased yield in organically managed soil with a high density of native AMF and fertilized with cow manure compost and magmatic Kola apatite. Hu et al. (2009) found that AMF inoculum enhanced maize P uptake and yield only in P-limited conditions, and not in soils receiving balanced fertilization. However, the effect of AMF inoculation is not always dependent on fertilization level. For example, there is some evidence that AMF inoculation can be beneficial for crop yield and P uptake under conventionally managed and even high fertilization levels (available P), possibly due to suppressed indigenous AMF community (Hamel et al., 1997; Kahiluoto and Vestberg, 1998). The overall challenge with AMF inoculation in field conditions is to find the most effective combination of AMF community with a host crop in specific conditions (Berruti et al., 2016; Gosling et al., 2006).

All three BM products tested (gBM, gBM-FM, and pyrBM) increased STP similarly to the SP150 treatment at the higher P level, indicating that soil P fertility was similarly improved and that these products can provide P for crops beyond the first growth period. According to Sneller and Laboski (2009), a substantial amount of P in pelleted poultry litter can be available for crops in the following year. However, taking into account the low availability of P in pyrBM in our pot experiment and the possible increase in P solubility in acidic conditions (Kratz et al., 2019), the acid ammonium acetate extraction (pH 4.65) used here for STP determination might have overestimated the short-term bioavailability of P in soils treated with pyrBM. This was also shown with meat and bone meal after four ryegrass harvests (Ylivainio et al., 2008), suggesting that also P derived from broiler manure products turns into more bioavailable form in a long term, as both materials contain mainly Ca associated P. For pyrBM, which contained substantial amounts of sparingly soluble P, further studies using an extended growing period and/or plants that can benefit from the long-term fertilizer effect are required, particularly if application rate is determined according to labile P content, or if P fertilizer is intended to be utilized over several growing seasons.

Knowledge of the bioavailability of P in organic fertilizers is essential for optimized fertilization according to crop needs, and thus minimizing unnecessary build-up of soil P and associated negative off-site effects. This study showed that granulation and pyrolysis produce poultry manure-based organic fertilizers with very distinct properties with respect to P bioavailability. Both granulated BM products could be suitable for annual crops, as they provide sufficient P from the beginning of the growing period. When amended with FM, gBM would also decrease the need for additional use of mineral N fertilizers, since FM addition increased total N content in the gBM product, and the mineral-N fertilizer equivalence of FM is higher than that of gBM (Keskinen et al., 2020). Pyrolyzed broiler manure could be used as a slow-release or storage P fertilizer. The possibilities to enhance P bioavailability in pyrolyzed manure by co-feedstocks (see Zwetsloot et al., 2015) should be studied further. As some N evaporates during pyrolysis and the remainder is transformed into sparsely available form (Keskinen et al., 2020), one viable option to optimize the nutrient content could be spiking broiler manure with FM after pyrolysis, followed by granulation to enable broadcasting of pyrBM with the same equipment as mineral fertilizers.

5. Conclusions

This study showed that broiler manure can be processed into organic fertilizer products for different purposes to replace mineral P fertilizers. Availability of P in granulated broiler manure to ryegrass during one growing season was comparable to that of mineral P fertilizer, whereas adding feather meal and pyrolysis decreased the yield-based mineral-P equivalence to around 75% (mean of both P levels) and 45%, respectively. However, based on P uptake, P in the feather meal-amended granulated broiler manure was comparable to that of mineral P fertilizer. Short-term (first harvest) mineral-P equivalence based on yield was 120%, 85% (mean of both P levels), and 75% for granulated unamended, granulated feather meal-amended, and pyrolyzed broiler manure, respectively. AMF inoculation was ineffective in improving P availability in pyrolyzed manure in a soil containing indigenous AMF.

CRediT authorship contribution statement

Minna Sarvi: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Marleena Hagner:** Conceptualization, Investigation, Writing - review & editing. **Sannakajsa Velmala:** Conceptualization, Investigation, Writing - review & editing. **Helena Soinne:** Conceptualization, Investigation, Writing - review & editing. **Risto Uusitalo:** Conceptualization, Writing - review & editing. **Riikka Keskinen:** Conceptualization, Writing - review & editing. **Kari Ylivainio:** Conceptualization, Writing - review & editing. **Janne Kaseva:** Conceptualization, Formal analysis, Writing - review & editing. **Kimmo Rasa:** Conceptualization, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2021.101584.

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